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15 May 1997 (15.05.97)

(54) Title: INHIBITION OF GENE EXPRESSION

(57) Abstract

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

INTERNATIONAL SEARCH REPORT

Interna... Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N15/79 C12N15/54 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 90 12084 A (DNA PLANT TECHN CORP) 18 October 1990 see page 9, line 17 see page 11, line 25 - page 12, line 11	1-7
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X	WO 92 14827 A (INST GENBIOLOGISCHE FORSCHUNG) 3 September 1992	19
A	see the whole document	1-10, 15-18, 20-22

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 December 1996	0 8. 04. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	MADDOX, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interna al Application No.
PCT/EP 96/03053

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 92 11375 A (AMYLOGENE HB) 9 July 1992 see the whole document	19 1-10, 15-18, 20-22
P,X	WO 95 26407 A (NAT STARCH CHEM INVEST ;COOKE DAVID (GB); GIDLEY MICHAEL JOHN (GB)) 5 October 1995 see page 15 - page 16	19
A	PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM ON PLANT POLYMERIC CARBOHYDRATES, no. 134, 1 July 1992, pages 33-39, XP002014045 WILLMITZER L ET AL: "STARCH SYNTHESIS IN TRANSGENIC PLANTS" see page 38	2
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 25, 5 September 1993, pages 19084-19091, XP002014043 MIZUNO K ET AL: "ALTERATION OF THE STRUCTURAL PROPERTIES OF STARCH COMPONENTS BY THE LACK OF AN ISOFORM OF STARCH BRANCHING ENZYME IN RICE SEEDS" see the whole document	2
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A	THEORETICAL AND APPLIED GENETICS, vol. 88, 1994, pages 369-375, XP002021173 FLIPSE, E., ET AL.: "Expression of a wild-type GBSS gene introduced into and amylose-free potato mutant by Agrobacterium tumefaciens and the inheritance of the inserts at the microsporic level" see page 374, left-hand column, paragraph 3 - right-hand column	1-3

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C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	MOLECULAR AND GENERAL GENETICS, vol. 246, no. 6, 20 March 1995, pages 745-755, XP000571429 KUIPERS A G J ET AL: "FACTORS AFFECTING THE INHIBITION BY ANTISENSE RNA OF GRANULE-BOUND STARCH SYNTHASE GENE EXPRESSION IN POTATO" see the whole document ---	1-3
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A	WO 92 15680 A (UNIV TEXAS) 17 September 1992 see page 5, line 14 - page 8, line 10 ---	1
A	CHEMICAL ABSTRACTS, vol. 121, no. 9, 29 August 1994 Columbus, Ohio, US; abstract no. 101242, SHIMADA, H., ET AL.: "Molecular cloning of cDNA and gene for rice starch branching enzyme and its use for breeding" XP002021261 see abstract & JP 06 098 656 A (MITSUI) 12 April 1994 ---	9
A	PLANT PHYSIOLOGY, vol. 107, March 1995, pages 679-685, XP002021174 MATZKE, M.A., ET AL.: "How and why do plants inactivate homologous (trans)genes?" see the whole document ---	1-10, 15-22
A	WO 94 09144 A (ZENECA LTD) 28 April 1994 see page 10, line 1-18 ---	15,22
A	WO 94 11520 A (ZENECA LTD ;KEELING PETER LEWIS (GB)) 26 May 1994 see claims 6,13 ---	15,22
A	US 4 740 463 A (WEINBERG ROBERT A ET AL) 26 April 1988 see column 8 - column 9 -----	15,22

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP 96/03053

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-10,15,20,21,22 (all completely) and 16-19 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 96/ 03053

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims: 1-10,15,20,21,22 (all completely) and 16-19 (all partially)
Method for affecting enzyme/protein activity using sense intron sequences, particularly affecting SBE, amylopectin levels or starch composition.

2. Claims: 11-14 (all completely) and 16-19 (all partially)
Method for tissue specific expression of transgenes using the SBE promoter of Seq.ID.14.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[illegible] Appli[illegible]
PCT/EP 96/0[illegible]

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna... Application No
PCT/EP 96/03053

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AOJP DATE 17/04/97 PCT NUMBER PCT/EP96/03053
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II

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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(54) Title: INHIBITION OF GENE EXPRESSION

(57) Abstract

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

INHIBITION OF GENE EXPRESSION

The present invention relates to a method of inhibiting gene expression, particularly inhibiting gene expression in a plant. The present invention also relates to a nucleotide sequence useful in the method. In addition, the present invention relates to a promoter that is useful for expressing the nucleotide sequence.

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylose consists essentially of straight chains of α-1-4-linked glycosyl residues. Amylopectin comprises chains of α-1-4-linked glycosyl residues with some α-1-6 branches. The branched nature of amylopectin is accomplished by the action of *inter alia* an enzyme commonly known as the starch branching enzyme ("SBE"). SBE catalyses the formation of branch points in the amylopectin molecule by adding α-1,4 glucans through α-1,6-glucosidic branching linkages. The biosynthesis of amylose and amylopectin is schematically shown in Figure 1, whereas the α-1-4-links and the α-1-6 links are shown in Figure 2.

It is known that starch is an important raw material. Starch is widely used in the food, paper, and chemical industries. However, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties.

Within the past few years it has become desirable to make genetically modified plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In this regard, June Bourque provides a detailed summary of antisense strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149).

Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences (for example see the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3) there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity.

According to a first aspect of the present invention there is provided a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence partially or completely codes (is) an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is a sense exon sequence normally associated with the intron.

According to a second aspect of the present invention there is provided a method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

According to a third aspect of the present invention there is provided a sequence comprising the nucleotide sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof.

According to a fourth aspect of the present invention there is provided a promoter comprising the sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

According to a fifth aspect of the present invention there is provided a construct capable of comprising or expressing the present invention.

According to a sixth aspect of the present invention there is provided a vector comprising or expressing the present invention.

According to a seventh aspect of the present invention there is provided a cell, tissue or organ comprising or expressing the present invention.

According to an eighth aspect of the present invention there is provided a transgenic starch producing organism comprising or expressing the present invention. According to a ninth aspect of the present invention there is provided a starch obtained from the present invention.

According to a tenth aspect of the present invention there is provided pBEA11 (NCIMB 40754). According to an eleventh aspect of the present invention there is provided a sense nucleotide sequence that is obtainable from λ-SBE 3.2 (NCIMB 40751) or λ-SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.

A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

Thus, the present invention provides a method of preparing tailor-made starches in plants which could replace the post-harvest modified starches.

Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a more beneficial effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity. With regard to this advantage of the present invention it is to be noted that there is some degree of homology between coding regions of SBEs. However, there is little or no homology with the intron sequences of SBEs. Thus, sense intron expression provides a mechanism to affect selectively the expression of a particular SBE. This advantageous aspect could be used, for example, to reduce or eliminate a particular SBE enzyme and replace that enzyme with another enzyme which can be another branching enzyme or even a recombinant version of the affected enzyme or even a hybrid enzyme which could for example comprise part of a SBE enzyme from one source and at least a part of another SBE enzyme from another source. This particular feature of the present invention is covered by the combination aspect of the present invention which is discussed in more detail later.

Thus the present invention provides a mechanism for selectively affecting SBE activity. This is in contrast to the prior art methods which are dependent on the use of for example antisense exon expression whereby it would not be possible to introduce new SBE activity without affecting that activity as well.

Preferably with the first aspect of the present invention starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

Preferably with the first or second aspect of the present invention the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

Preferably with the fourth aspect of the present invention the promoter is in combination with a gene of interest ("GOI").

Preferably the enzymatic activity is reduced or eliminated.

Preferably the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

Preferably the nucleotide sequence codes, partially or completely, for two or more introns and wherein each intron is in a sense orientation.

Preferably the nucleotide sequence comprises at least 350 nucleotides (e.g. 350 bp), more preferably at least 500 nucleotides (e.g. 500 bp).

Preferably the nucleotide sequence comprises the sequence shown as any one of SEQ. I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

Preferably the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ. I.D. No. 14 or a variant, derivative or homologue thereof.

Preferably the transgenic starch producing organism is a plant.

A preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron and wherein starch branching enzyme activity is affected and/or the levels of amylose are altered and/or the composition of starch is changed.

A more preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation: wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron: wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed: and wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

The term "nucleotide" in relation to the present invention includes DNA and RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant DNA techniques.

The term "intron" is used in its normal sense as meaning a segment of nucleotides, usually DNA, that does not encode part or all of an expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides, usually DNA, encoding part or all of an expressed protein or enzyme.

Thus, the term "intron" refers to gene regions that are transcribed into RNA molecules, but which are spliced out of the RNA before the RNA is translated into a protein In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as any one of

the sense sequences shown as SEQ.I.D. No.s 1-13. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

Likewise, the terms "variant" or "homologue" or "fragment" in relation to the promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective promoter sequence providing the resultant promoter sequence allows expression of a GOI, preferably wherein the resultant promoter sequence has at least the same effect as SEQ.I.D. No. 14. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide sequence according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

The intron sequence of the present invention can be any one or all of the intron sequences of the present invention, including partial sequences thereof, provided that if partial sense sequences are used (i.e. sequences that are not or do not comprise any one or more of the full sequences shown as SEQ.I.D. No.1-13) the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences that are shorter than any one of the full sense sequences shown as SEQ.I.D.No.s 1 to 13 but which comprise nucleotides that are adjacent the respective exon or exons.

With regard to the second aspect of the present invention (i.e. specifically affecting SBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of the SBE gene (but not sense exon sequences naturally associated with the intron sequence), including complete or partial sequences thereof, providing the nucleotide sequences can affect SBE activity, preferably wherein the nucleotide sequences reduce or eliminate SBE activity. Preferably, the nucleotide sequence of the second aspect of the present invention does not comprise sense exon sequences.

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E.Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the sense nucleotide sequence aspect of the present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. The terms do not cover the natural combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence of the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. In one aspect, preferably the promoter is the patatin promoter or the E35S promoter. In another aspect, preferably the promoter is the SBE promoter.

If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. By way of example, the promoter for the nucleotide sequence of the present invention can be the α-Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the α-Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. Alternatively, the promoter for the nucleotide sequence of the present invention can be the α-Amy 3 promoter (otherwise known as the Amy 3

promoter, the Amy 351 promoter or the α-Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994.

The present invention also encompasses the use of a promoter to express a nucleotide sequence according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a promoter in some instances is advantageous. In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses the nucleotide sequence of the present invention in a more specific manner such as in just one specific tissue type or organ. The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a gene coding for the enzyme of the present invention in at least one (but not all) specific tissue of the original promoter. Examples of partial inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part. Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

The construct and/or the vector of the present invention may include a transcriptional termination region.

The nucleotide according to the present invention can be expressed in combination (but not necessarily at the same time) with an additional construct. Thus the present invention also provides a combination of constructs comprising a first construct comprising the nucleotide sequence according to the present invention operatively

linked to a first promoter: and a second construct comprising a GOI operatively linked to a second promoter (which need not be the same as the first promoter). With this aspect of the present invention the combination of constructs may be present in the same vector, plasmid, cells, tissue, organ or organism. This aspect of the present invention also covers methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a plant. With this aspect of the present invention the second construct does not cover the natural combination of the gene coding for an enzyme ordinarily associated with the wild type gene promoter when they are both in their natural environment.

An example of a suitable combination would be a first construct comprising the nucleotide sequence of the present invention and a promoter, such as the promoter of the present invention, and a second construct comprising a promoter, such as the promoter of the present invention, and a GOI wherein the GOI codes for another starch branching enzyme either in sense or antisense orientation.

The above comments relating to the term "construct" for the sense nucleotide aspect of the present invention are equally applicable to the term "construct" for the promoter aspect of the present invention. In this regard, the term includes the promoter according to the present invention directly or indirectly attached to a GOI.

The term "GOI" with reference to the promoter aspect of the present invention or the combination aspect of the present invention means any gene of interest, which need not necessarily code for a protein or an enzyme - as is explained later. A GOI can be any nucleotide sequence that is either foreign or natural to the organism in question, for example a plant.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. An example of such a GOI is the nucleotide sequence according to the present invention.

The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and α-galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for α-amylase, a protease or a glucanase. Alternatively, the GOI can be a nucleotide sequence according to the present invention.

The GOI can be the nucleotide sequence coding for the arabinofuranosidase enzyme which is the subject of our co-pending UK patent application 9505479.7. The GOI can be the nucleotide sequence coding for the glucanase enzyme which is the subject of our co-pending UK patent application 9505475.5. The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9413439.2. The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9421290.9. The GOI can be any of the nucleotide sequences coding for the α-glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397.

In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase. Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

As mentioned above, the present invention provides a mechanism for selectively affecting a particular enzymatic activity.

In an important application of the present invention it is now possible to reduce or eliminate expression of a genomic nucleotide sequence coding for a genomic protein or enzyme by expressing a sense intron construct for that particular genomic protein or enzyme and (e.g. at the same time) expressing a recombinant version of that enzyme or protein - in other words the GOI is a recombinant nucleotide sequence coding for the genomic enzyme or protein. This application allows expression of desired recombinant enzymes and proteins in the absence of (or reduced levels of) respective genomic enzymes and proteins. Thus the desired recombinant enzymes and proteins can be easily separated and purified from the host organism. This particular aspect of the present invention is very advantageous over the prior art methods which, for example, rely on the use of anti-sense exon expression which methods also affect expression of the recombinant enzyme.

Thus, a further aspect of the present invention relates to a method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron. Additional aspects cover the combination of those nucleotide sequences including their incorporation in constructs, vectors, cells, tissues and transgenic organisms.

Therefore the present invention also relates to a combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding the enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

The GOI may even code for one or more introns but in an antisense orientation, such as any one or more of the antisense intron sequences presented in the attached sequence listings. For example, the present invention also covers the expression of for example a sense intron (e.g. SEQ.I.D.No. 1) in combination with for example an antisense sense intron which preferably is not complementary to the sense intron sequence (e.g. SEQ.I.D.No. 16).

The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cereals such as wheat, maize, and barley; fruit; trees; flowers; and other plant crops. Preferably, the term means "potato".

The term "transgenic organism' in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products obtained therefrom, and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide sequence of the present invention is incorporated in the genome of the organism. Preferably the transgenic organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Sambrook *et al*. in Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

Even though the enzyme according to the present invention and the nucleotide sequence coding for same are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is

capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant.

The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.* (1980). Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.* (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

The nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appears to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof. As these plasmids are well-known and widely employed in the construction of transgenic

plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the nucleotide sequence or construct of the present invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the present invention, which DNA is subsequently transferred into the plant cell to be modified.

If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An *et al.*, EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists*, eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be performed in or on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the GOI (such as the nucleotide sequence according to the present invention) and, optionally, a promoter, a plant to be infected is wounded, e.g. by cutting the plant with a razor blade or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In this way, the nucleotide or construct of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is then used for the transformation in *E coli*. The *E.coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same

- or different plasmid.

After the introduction of the nucleotide sequence or construct according to the present invention in the plants the presence and/or insertion of further DNA sequences may be necessary - such as to create combination systems as outlined above (e.g. an organism comprising a combination of constructs).

The above commentary for the transformation of prokaryotic organisms and plants with the nucleotide sequence of the present invention is equally applicable for the transformation of those organisms with the promoter of the present invention.

In summation, the present invention relates to affecting enzyme activity by expressing sense intron sequences.

Also, the present invention relates to a promoter useful for the expression of those sense intron sequences.

The following samples have been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 13 July 1995:

NCIMB 40754 (which refers to pBEA 11 as described herein);

NCIMB 40751 (which refers to λ-SBE 3.2 as described herein), and

NCIMB 40752 (which refers to λ-SBE 3.4 as described herein).

A highly preferred embodiment of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely,

for an intron in a sense orientation: wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron: wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed: and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754 or a variant, derivative or homologue thereof.

A more highly preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation: wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron: wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed: wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof; and wherein the intron nucleotide sequence is obtainable from NCIMB 40751, NCIMB 40752, or NCIMB 40754, or a variant, derivative or homologue thereof.

The present invention will now be described only by way of example, in which reference is made to the following attached Figures:

Figure 1, which is a schematic representation of the biosynthesis of amylose and amylopectin:

Figure 2, which is a diagrammatic representation of the α-1-4-links and the α-1-6 links of amylopectin:

Figure 3, which is a diagrammatic representation of the exon-intron structure of a genomic SBE clone:

Figure 4, which is a plasmid map of pPATA1, which is 3936 bp in size;

Figure 5, which is a plasmid map of pABE7, which is 5106 bp in size;

Figure 6, which is a plasmid map of pVictorIV Man, which is 7080 bp in size;

Figure 7, which is a plasmid map of pBEA11, which is 9.54 kb in size;

Figure 8, which shows the full genomic nucleotide sequence for SBE including the promoter, exons and introns;

Figure 9, which is a plasmid map of pVictor5a, which is 9.12 kb in size; and

Figure 10, which is a plasmid map of pBEP2, which is 10.32 kb in size.

Figures 1 and 2 were referred to above in the introductory description concerning starch in general. As mentioned, Figure 3 is a diagrammatic representation of the exon-intron structure of a genomic SBE clone, the sequence of which is shown in Figure 8. This clone, which has about 11.5 k base pairs, comprises 14 exons and 13 introns. The introns are numbered in increasing order from the 5' end to the 3' end and correspond to SEQ.I.D.No.s 1-13, respectively. Their respective antisense intron sequences are shown as SEQ.I.D.No.s 15-27.

In more detail, Figures 3 and 8 present information on the 11468 base pairs of a potato SBE gene. The 5' region from nucleotides 1 to 2082 contain the promoter region of the SBE gene. A TATA box candidate at nucleotide 2048 to 2051 is boxed. The homology between a potato SBE cDNA clone (Poulsen & Kreiberg (1993) Plant Physiol 102: 1053-1054) and the exon DNAs begin at 2083 bp and end at 9666 bp. The homology between the cDNA and the exon DNA is indicated by nucleotides in upper case letters, while the translated amino acid sequences are shown in the single letter code below the exon DNA. Intron sequences are indicated by lower case letters.

Figure 7 is a plasmid map of pBEA7, which is 9.54 k base pairs in size. Plasmid pBEA 11 comprises the first intron sequence of the potato SBE gene. This first intron sequence, which has 1177 base pairs, is shown in Figure 3 and lies between the first exon and the second exon.

These experiments and aspects of the present invention are now discussed in more detail.

EXPERIMENTAL PROTOCOL

ISOLATION, SUBCLONING IN PLASMIDS, AND SEQUENCING OF GENOMIC SBE CLONES

Various clones containing the potato SBE gene were isolated from a Desiree potato genomic library (Clontech Laboratories Inc., Palo Alto CA, USA) using radioactively labelled potato SBE cDNA (Poulsen & Kreiberg (1993) Plant Physiol. 102:1053-1054) as probe. The fragments of the isolated λ-phages containing SBE DNA (λSBE 3.2 - NCIMB 40751 - and λSBE-3.4 - NCIMB 40752) were identified by Southern analysis and then subcloned into pBluescript II vectors (Clontech Laboratories Inc., Palo Alto CA, USA). λSBE 3.2 contains a 15 kb potato DNA insert and λSBE-3.4 contains a 13 kb potato DNA insert. The resultant plasmids were called pGB3, pGB11, pGB15, pGB16 and pGB25 (see discussion below). The respective inserts were then sequenced using the Pharmacia Autoread Sequencing Kit (Pharmacia, Uppsala) and a A.L.F. DNA sequencer (Pharmacia, Uppsala).

In total, a stretch of 11.5 kb of the SBE gene was sequenced. The sequence was deduced from the above-mentioned plasmids, wherein: pGB25 contains the sequences from 1 bp to 836 bp, pGB15 contains the sequences from 735 bp to 2580 bp, pGB16 contains the sequences from 2580 bp to 5093 bp, pGB11 contains the sequences from 3348 bp to 7975 bp, and pGB3 contains the sequences from 7533 bp to 11468 bp.

In more detail, pGB3 was constructed by insertion of a 4 kb *EcoRI* fragment isolated from λSBE 3.2 into the *EcoRI* site of pBluescript II SK (+). pGB11 was constructed by insertion of a 4.7 kb *XhoI* fragment isolated from λSBE 3.4 into the *XhoI* site of pBluescript II SK (+). pGB15 was constructed by insertion of a 1.7 kb *SpeI* fragment isolated from λSBE 3.4 into the *SpeI* site of pBluescript II SK (+). pGB16 was constructed by insertion of a 2.5 kb *SpeI* fragment isolated from λSBE 3.4 into the *SpeI* site of pBluescript II SK (+). For the construction of pGB25 a PCR fragment was produced with the primers

5' GGA ATT CCA GTC GCA GTC TAC ATT AC 3'

and

5' CGG GAT CCA GAG GCA TTA AGA TTT CTG G 3'

and λSBE 3.4 as a template.

The PCR fragment was digested with *BamHI* and *EcoRI*, and inserted in pBluescript II SK (+) digested with the same restriction enzymes.

CONSTRUCTION OF PLASMID pBEA11

The SBE intron 1 was amplified by PCR using the oligonucleotides

5' CGG GAT CCA AAG AAA TTC TCG AGG TTA CAT GG 3'

and

5' CGG GAT CCG GGG TAA TTT TTA CTA ATT TCA TG 3'

and the λSBE 3.4 phage containing the SBE gene as template.

The PCR product was digested with *BamH*I and inserted in a sense orientation in the *BamH*I site of plasmid pPATA1 (described in WO 94/24292) between the patatin promoter and the 35S terminator. This construction, pABE7, was digested with *Kpn*I, and the 2.4 kb "patatin promoter-SBE intron i- 35S terminator" *Kpn*I fragment was isolated and inserted in the *Kpn*I site of the plant transformation vector pVictorIV Man yielding plasmid pBEA11.

PRODUCTION OF TRANSGENIC POTATO PLANTS

Axenic stock cultures

Shoot cultures of *Solanum tuberosum* 'Bintje' and 'Dianella' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), Physiol. Plant. 18: 100-127, in addition containing 2 μM silver thiosulphate at 25°C and 16 h light/8 h dark.

The cultures were subcultured after approximately 40 days. Leaves were then cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) were cut into internodal segments (approximately 0.8 cm). The segments were placed into liquid LS-substrate containing the transformed *Agrobacterium tumefaciens* containing the binary vector of interest. The *Agrobacterium* were grown overnight in YMB-substrate (di-potassium hydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the *Agrobacterium* strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments were left in the suspension of *Agrobacterium* for 30 minutes and then the excess of bacteria were removed by blotting the segments on sterile filter paper.

Co-cultivation

The shoot segments were co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants were covered with sterile filter papers, and the petri dishes were placed at 25°C and 16 h light/ 8 dark.

"Washing" procedure

After the 48 h on the co-cultivation substrate the segments were transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers were gently shaken and by this procedure the major part of the *Agrobacterium* was either washed off the segments and/or killed.

Selection

After the washing procedure the segments were transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used. The segments were sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continued for 3-4 months.

Rooting of regenerated shoots

The regenerated shoots were transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l).

The transgenic genotype of the regenerated shoot were verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al. Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993. NAR 21 pp 4153-4154). Plants which were not positive in any of these assays were discarded or used as controls. Alternatively, the transgenic plants could be verified by performing a GUS assay on the co-introduced β-glucuronidase gene according to Hodal, L. *et al.* (Pl. Sci. (1992), 87: 115-122).

Transfer to soil

The newly rooted plants (height approx. 2-3 cms) were transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m^2/sec). When the plants were well established they were transferred to the greenhouse, where they were grown until tubers had developed and the upper part of the plants were senescing.

Harvesting

The potatoes were harvested after about 3 months and then analysed.

BRANCHING ENZYME ANALYSIS

The SBE expression in the transgenic potato lines were measured using the SBE assays described by Blennow and Johansson (Phytochemistry (1991) 30:437-444) and by standard Western procedures using antibodies directed against potato SBE.

STARCH ANALYSIS

Starch was isolated from potato tubers and analysed for the amylose:amylopectin ratio (Hovenkamp-Hermelink et al. (1988) Potato Research 31:241-246). In addition, the chain length distribution of amylopectin was determined by analysis of isoamylase

digested starch on a Dionex HPAEC. The number of reducing ends in isoamylase digested starch was determined by the method described by N. Nelson (1944) J. Biol.Chem. 153:375-380.

The results revealed that there was a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch SBE in the transgenic plants.

CONSTRUCTION OF SBE PROMOTER CONSTRUCT

An SBE promoter fragment was amplified from λ-SBE 3.4 using primers:

5' CCA TCG ATA CTT TAA GTG ATT TGA TGG C 3'

and

5' CGG GAT CCT GTT CTG ATT CTT GAT TTC C 3'.

The PCR product was digested with *Cla*I and *BamH*I. The resultant 1.2 kb fragment was then inserted in pVictor5a (see Figure 9) linearised with *Cla*I and *Bgl*II yielding pBEP2 (see Figure 10).

STARCH BRANCHING ENZYME MEASUREMENTS OF POTATO TUBERS

Potatoes from potato plants transformed with pBEA11 were cut in small pieces and homogenised in extraction buffer (50 mM Tris-HCl pH 7.5, Sodium-dithionit (0.1 g/l), and 2 mM DTT) using a Ultra-Turax homogenizer; 1 g of Dowex xl. was added pr. 10 g of tuber. The crude homogenate was filtered through a miracloth filter and centrifuged at 4°C for 10 minutes at 24.700 g. The supernatant was used for starch branching enzyme assays.

The starch branching enzyme assays were carried out at 25 oC in a volume of 400 µl composed of 0.1 M Na citrate buffer pH 7.0, 0.75 mg/ml amylose, 5 mg/ml bovine serum albumin and the potato extract. At 0, 15 30 and 60 minutes aliqouts of 50 µl were removed from the reaction into 20 µl 3 N HCl. 1 ml of iodine solution was added and the decrease in absorbance at 620 nm was measured with an ELISA spectrophotometer.

The starch branching enzyme (SBE) levels in tuber extracts were measured from 24 transgenic Dianella potato plants transformed with plasmid pBEA11.

The results showed that the BEA11 transgenic lines produced tubers which have SBE levels that are only 10 % to 15 % of the SBE levels found in non transformed Dianella plants.

SUMMATION

The above-mentioned examples relate to the isolation and sequencing of a gene for potato SBE. The examples further demonstrate that it is possible to prepare SBE intron constructs. These SBE intron constructs can be introduced into plants, such as potato plants. After introduction, a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch in plants can be achieved.

Without wishing to be bound by theory it is believed that the expressed sense intron nucleotide sequence according to the present invention affects enzymatic activity *via* co-suppression and/or trans-activation. Reviews of these mechanisms has been published by Finnegan and McElroy (1994 Biotechnology 12 pp 883 - 887) and Matzke and Matzke (1995 TIG 11 No. 1 pp 1 - 3). By these mechanisms, it is believed that the sense introns of the present invention reduce the level of plant enzyme activity (in particular SBE activity), which in turn for SBE activity is believed to influence the amylose:amylopectin ratio and thus the branching pattern of amylopectin.

Thus, the present invention provides a method wherein it is possible to manipulate the starch composition in plants, or tissues or cells thereof, such as potato tubers, by reducing the level of SBE activity by using sense intron sequences.

In summation the present invention therefore relates to the surprising use of sense intron sequences in a method to affect enzymatic activity in plants.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention. For example, it may be possible to use antisense promoter sequences to affect enzymatic activity, such as antisense SBE promoter - such as a nucleotide sequence comprising the nucleotide sequence shown as SEQ. I.D. No. 28 or a variant, derivative or homologue thereof.

The following pages present a number of sequence listings which have been consecutively numbered from SEQ.I.D. No. 1 - SEQ.I.D. No. 29. In brief, SEQ.I.D. No. 1 - SEQ.I.D. No. 13 represent sense intron sequences (genomic DNA); SEQ.I.D. No. 14 represents the SBE promoter sequence (genomic sequence); SEQ.I.D. No. 15 - SEQ.I.D. No. 27 represent antisense intron sequences; and SEQ. I.D. No. 28 represents the sequence complementary to the SBE promoter sequence - i.e. the SBE promoter sequence in antisense orientation. The full genomic nucleotide sequence for SBE including the promoter, exons and introns is shown as SEQ. I.D. No. 29 (see Figures 3 and 8 which highlight particular gene features).

SEQUENCE INFORMATION

SEQ.I.D. No. 1
Intron 1 sequence (1167 bp).

GTAATTTTTACTAATTTCATGTTAATTTCAATTATTTTTAGCCTTTGCATTTCATTTTCCAATATATCT
GGATCATCTCCTTAGTTTTTTATTTTATTTTTTATAATATCAAATATGGAAGAAAAATGACACTTGTAG
AGCCATATGTAAGTATCATGTGACAAATTTGCAAGGTGGTTGAGTGTATAAAATTCAAAAATTGAGAGA
TGGAGGGGGGGTGGGGGBARAGACAATATTTAGAAAGAGTGTTCTAGGAGGTTATGGAGGACACGGATG
AGGGGTAGAAGGTTAGTTAGGTATTTGAGTGTTGTCTGGCTTATCCTTTCATACTAGTAGTCGTGGAAT
TATTTGGGTAGTTTCTTGTTTTGTTATTTGATCTTTGTTATTCTATTTTCTGTTTCTTGTACTTCGATT
ATTGTATTATATATCTTGTCGTAGTTATTGTTCCTCGGTAAGAATGCTCTAGCATGCTTCCTTTAGTGT
TTTATCATGCCTTCTTTATATTCGCGTTGCTTTGAAATGCTTTTACTTTAGCCGAGGGTCTATTAGAAA
CAATCTCTCTATCTCGTAAGGTAGGGGTAAAGTCCTCACCACACTCCACTTGTGGGATTACATTGTGTT
TGTTGTTGTAAATCAATTATGTATACATAATAAGTGGATTTTTTACAACACAAATACATGGTCAAGGGC
AAAGTTCTGAACACATAAAGGGTTCATTATATGTCCAGGGATATGATAAAAATTGTTTCTTTGTGAAAG
TTATATAAGATTTGTTATGGCTTTTGCTGGAAACATAATAAGTTATAATGCTGAGATAGCTACTGAAGT
TTGTTTTTTCTAGCCTTTTAAATGTACCAATAATAGATTCCGTATCGAACGAGTATGTTTTGATTACCT
GGTCATGATGTTTCTATTTTTTACATTTTTTTGGTGTTGAACTGCAATTGAAAATGTTGTATCCTATGA
GACGGATAGTTGAGAATGTGTTCTTTGTATGGACCTTGAGAAGCTCAAACGCTACTCCAATAATTTCTA
TGAATTCAAATTCAGTTTATGGCTACCAGTCAGTCCAGAAATTAGGATATGCTGCATATACTTGTTCAA
TTATACTGTAAAATTTCTTAAGTTCTCAAGATATCCATGTAACCTCGAGAATTTCTTTGACAG

SEQ.I.D. No. 2
Intron 2 sequence (321 bp).

GTATGTTTGATAATTTATATGGTTGCATGGATAGTATATAAATAGTTGGAAAACTTCTGGACTGGTGCT
CATGGCATATTTGATCTGTGCACCGTGTGGAGATGTCAAACATGTGTTACTTCGTTCCGCCAATTTATA
ATACCTTAACTTGGGAAAGACAGCTCTTTACTCCTGTGGGCATTTGTTATTTGAATTACAATCTTTATG
AGCATGGTGTTTTCACATTATCAACTTCTTTCATGTGGTATATAACAGTTTTTAGCTCCGTTAATACCT
TTCTTCTTTTTGATATAAACTAACTGTGGTGCATTGCTTGCBKKK

SEQ.I.D. No. 3
Intron 3 sequence (504 bp).

GTAACAGCCAAAAGTTGTGCTTTAGGCAGTTTGACCTTATTTTGGAAGATGAATTGTTTATACCTACTT
TGACTTTGCTAGAGAATTTTGCATACCGGGGAGTAAGTAGTGGCTCCATTTAGGTGGCACCTGGCCATT
TTTTTGATCTTTTAAAAAGCTGTTTGATTGGGTCTTCAAAAAAGTAGACAAGGTTTTTGGAGAAGTGAC
ACACCCCCGGAGTGTCAGTGGCAAAGCAAAGATTTTCACTAAGGAGATTCAAAATATAAAAAAAGTATA
GACATAAACAAGCTGAGGGGATTCAACATGTACTATACAAGCATCAAATATAGTCTTAAAGCAATTTTG
TAGAAATAAAGAAAGTCTTCCTTCTGTTGCTTCACAATTTCCTTCTATTATCATGAGTTACTCTTTCTG
TTCGAAATAGCTTCCTTAATATTAAATTCATGATACTTTTGTTGAGATTTAGCAGTTTTTTCTTGTGTA
AACTGCTCTCTTTTTTTGCAG

SEQ.I.D. No. 4
Intron 4 sequence (146 bp).

GTAGGTCCTCGTCTACTACAAAATAGTAGTTTCCATCATCATAACAGATTTTCCTATTAAAGCATGATG
TTGCAGCATCATTGGCTTTCTTACATGTTCTAATTGCTATTAAGGTTATGCTTCTAATTAACTCATCCA
CAATGCAG

SEQ.I.D. No. 5
Intron 5 sequence (218 bp).

GTTTTGTTATTCATACCTTGAAGCTGAATTTTGAACACCATCATCACAGGCATTTCGATTCATGTTCTT
ACTAGTCTTGTTATGTAAGACATTTTGAAATGCAAAAGTTAAAATAATTGTGTCTTTACTAATTTGGAC
TTGATCCCATACTCTTTCCCTTAACAAAATGAGTCAATTCTATAAGTGCTTGAGAACTTACTACTTCAG
CAATTAAACAG

SEQ.I.D. No. 6
Intron 6 sequence (198 bp).

GTATTTTAAATTTATTTCTACAACTAAATAATTCTCAGAACAATTGTTAGATAGAATCCAAATATATAC
GTCCTGAAAGTATAAAAGTACTTATTTTCGCCATGGGCCTTCAGAATATTGGTAGCCGCTGAATATCAT
GATAAGTTATTTATCCAGTGACATTTTTATGTTCACTCCTATTATGTCTGCTGGATACAG

SEQ.I.D. No. 7
Intron 7 sequence (208bp)

GTTTGTCTGTTTCTATTGCATTTTAAGGTTCATATAGGTTAGCCACGGAAAATCTCACTCTTTGTGAGG
TAACCAGGGTTCTGATGGATTATTCAATTTTCTCGTTTATCATTTGTTTATTCTTTTCATGCATTGTGT
TTCTTTTTCAATATCCCTCTTATTTGGAGGTAATTTTTCTCATCTATTCACTTTTAGCTTCTAACCACAG

SEQ.I.D. No. 8
Intron 8 sequence (293 bp).

GTATGTCTTACATCTTTAGATATTTTGTGATAATTACAATTAGTTTGGCTTACTTGAACAAGATTCATT
CCTCAAAATGACCTGAACTGTTGAACATCAAAGGGGTTGAAACATAGAGGAAAACAACATGATGAATGT
TTCCATTGTCTAGGGATTTCTATTATGTTGCTGAGAACAAATGTCATCTTAAAAAAAACATTGTTTACT
TTTTTGTAGTATAGAAGATTACTGTATAGAGTTTGCAAGTGTGTCTGTTTTGGAGTAATTGTGAAATGT
TTGATGAACTTGTACAG

SEQ.I.D. No. 9
Intron 9 sequence (376 bp).

GTTCAAGTATTTTGAATCGCAGCTTGTTAAATAATCTAGTAATTTTTAGATTGCTTACTTGGAAGTCTA
CTTGGTTCTGGGGATGATAGCTCATTTCATCTTGTTCTACTTATTTTCCAACCGAATTTCTGATTTTTG
TTTCGAGATCCAAGTATTAGATTCATTTACACTTATTACCGCCTCATTTCTACCACTAAGGCCTTGATG
AGCAGCTTAAGTTGATTCTTTGAAGCTATAGTTTCAGGCTACCAATCCACAGCCTGCTATATTTGTTGG

ATACTTACCTTTTCTTTACAATGAAGTGATACTAATTGAAATGGTCTAAATCTGATATCTATATTTCTC
CGTCTTTCCTCCCCCTCATGATGAAATGCAG

SEQ.I.D. No. 10
Intron 10 sequence (172 bp).

GTAAAATCATCTAAAGTTGAAAGTGTTGGGTTTATGAAGTGCTTTAATTCTATCCAAGGACAAGTAGAA
ACCTTTTTACCTTCCATTTCTTGATGATGGATTTCATATTATTTAATCCAATAGCTGGTCAAATTCGGT
AATAGCTGTACTGATTAGTTACTTCACTTTGCAG

SEQ.I.D. No. 11
Intron 11 sequence (145 bp).

GTATATATGTTTTACTTATCCATGAAATTATTGCTCTGCTTGTTTTTAATGTACTGAACAAGTTTTATG
GAGAAGTAACTGAAACAAATCATTTTCACATTGTCTAATTTAACTCTTTTTTCTGATCCTCGCATGACG
AAAACAG

SEQ.I.D. No. 12
Intron 12 sequence (242 bp).
GTAAGGATTTGCTTGAATAACTTTTGATAATAAGATAACAGATGTAGGGTACAGTTCTCTCACCAAAAA
GAACTGTAATTGTCTCATCCATCTTTAGTTGTATAAGATATCCGACTGTCTGAGTTCGGAAGTGTTTGA
GCCTCCTGCCCTCCCCCTGCGTTGTTTAGCTAATTCAAAAAGGAGAAAACTGTTTATTGATGATCTTTG
TCTTCATGCTGACATACAATCTGTTCTCATGACAG

SEQ.I.D. No. 13
Intron 13 sequence (797 bp).

GTACAGTTCTTGCCGTGTGACCTCCCTTTTTATTGTGGTTTTGTTCATAGTTATTTGAATGCGATAGAA
GTTAACTATTGATTACCGCCACAATCGCCAGTTAAGTCCTCTGAACTACTAATTTGAAAGGTAGGAATA
GCCGTAATAAGGTCTACTTTTGGCATCTTACTGTTACAAAACAAAAGGATGCCAAAAAAATTCTTCTCT
ATCCTCTTTTTCCCTAAACCAGTGCATGTAGCTTGCACCTGCATAAACTTAGGTAAATGATCAAAAATG
AAGTTGATGGGAACTTAAAACCGCCCTGAAGTAAAGCTAGGAATAGTCATATAATGTCCACCTTTGGTG
TCTGCGCTAACATCAACAACAACATACCTCGTGTAGTCCCACAAAGTGGTTTCAGGGGGAGGGTAGAGT
GTATGCAAAACTTACTCCTATCTCAGAGGTAGAGAGGATTTTTTCAATAGACCCTTGGCTCAAGAAAAA
AAGTCCAAAAAGAAGTAACAGAAGTGAAAGCAACATGTGTAGCTAAAGCGACCCAACTTGTTTGGGACT
GAAGTAGTTGTTGTTGTTGAAACAGTGCATGTAGATGAACACATGTCAGAAAATGGACAACACAGTTAT
TTTGTGCAAGTCAAAAAAATGTACTACTATTTCTTTGTGCAGCTTTATGTATAGAAAAGTTAAATAACT
AATGAATTTTGCTAGCAGAAAAATAGCTTGGAGAGAAATTTTTTATATTGAACTAAGCTAACTATATTC
ATCTTTCTTTTTGCTTCTTCTTCTCCTTGTTTGTGAAG

SEQ.I.D. No. 14
DNA sequence of the SBE gene promoter region.

ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTCAGATTCTTTCGAGTTCTCAT 60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAACTGTTGCATCTGCTTCTTAGAACT 120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTTTCAAACTCTT 180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTTTCCTCAATGATGTTTACAGTGTTGTG 240
TTGTCATCTGTACTTTTGCCTATTACTTGTTTTGAGTTACATGTTAAAAAAGTGTTTATT 300
TTGCCATATTTTGTTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA 360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA 420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG 480
CATAAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC 540
AACGTTAATTTAGTAATTTTTGTTACGTACGTATATGAAATATTGAATTAATCACATGAA 600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT 660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAAAATAACTCTCATTTCAAGT 720
GGGATTAGAACTAGTTATTAAAAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT 780
AAAGTTTTTCATTTCATGCTAAAATTGTTAATTATTGTAATGTAGACTGCGACTGGAATT 840
ATTATAGTGTAAATTTATGCATTCAGTGTAAAATTAAAGTATTGAACTTGTCTGTTTTAG 900
AAAATACTTTATACTTTAATATAGGATTTTGTCATGCGAATTTAAATTAATCGATATTGA 960
ACACGGAATACCAAAATTAAAAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG 1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAGAATAAACTGACAAATTAATTTCTTTT 1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT 1140
GAATGATATTCATTTTTCATCCCATAAGTTCAATTTGATTGTCATACCACCCATGATGTT 1200
CTGAAAAATGCTTGGCCATTCACAAAGTTTATCTTAGTTCCTATGAACTTTATAAGAAGC 1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA 1320
TTAATATTGTAACTTTGTAATTGAGTGTGTCTACATCTTATTCAATCATTTAAGGTCATT 1380
AAAATAAATTATTTTTTGACATTCTAAAACTTTAAGCAGAATAAATAGTTTATCAATTAT 1440
TAAAAACAAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT 1500
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTTT 1560
TTTAGCCTAACCAACGAATATTTGTAAACTCACAACTTGATTAAAAGGGATTTACAACAA 1620
GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAATTTGGAGGTCAAAATTT 1680
TACCATAATCATTTGTATTTATAATTAAATTTTAAATATCTTATTTATACATATCTAGTA 1740
AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTCATATTAGGTCAATA 1800
AATCCTTAACTATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA 1860
CATGTATTATGTATACAAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA 1920
AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAAATATGTTTTACTTCAATTTCG 1980
AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT 2040
GTTTTTTTATAAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA 2100
GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTTATCAAA 2160
ACCCATTCG

SEQ.I.D. No. 15
Intron 1 antisense sequence (1167 bp).

CTGTCAAAGAAATTCTCGAGGTTACATGGATATCTTGAGAACTTAAGAAATTTTACAGTATAATTGAAC
AAGTATATGCAGCATATCCTAATTTCTGGACTGACTGGTAGCCATAAACTGAATTTGAATTCATAGAAA
TTATTGGAGTAGCGTTTGAGCTTCTCAAGGTCCATACAAAGAACACATTCTCAACTATCCGTCTCATAG
GATACAACATTTTCAATTGCAGTTCAACACCAAAAAAATGTAAAAAATAGAAACATCATGACCAGGTAA
TCAAAACATACTCGTTCGATACGGAATCTATTATTGGTACATTTAAAAGGCTAGAAAAAACAAACTTCA
GTAGCTATCTCAGCATTATAACTTATTATGTTTCCAGCAAAAGCCATAACAAATCTTATATAACTTTCA
CAAAGAAACAATTTTTATCATATCCCTGGACATATAATGAACCCTTTATGTGTTCAGAACTTTGCCCTT
GACCATGTATTTGTGTTGTAAAAAATCCACTTATTATGTATACATAATTGATTTACAACAACAAACACA
ATGTAATCCCACAAGTGGAGTGTGGTGAGGACTTTACCCCTACCTTACGAGATAGAGAGATTGTTTCTA
ATAGACCCTCGGCTAAAGTAAAAGCATTTCAAAGCAACGCGAATATAAAGAAGGCATGATAAAACACTA
AAGGAAGCATGCTAGAGCATTCTTACCGAGGAACAATAACTACGACAAGATATATAATACAATAATCGA
AGTACAAGAAACAGAAAATAGAATAACAAAGATCAAATAACAAAACAAGAAACTACCCAAATAATTCCA
CGACTACTAGTATGAAAGGATAAGCCAGACAACACTCAAATACCTAACTAACCTTCTACCCCTCATCCG
TGTCCTCCATAACCTCCTAGAACACTCTTTCTAAATATTGTCTYTVCCCCCACCCCCCCTCCATCTCTC
AATTTTTGAATTTTATACACTCAACCACCTTGCAAATTTGTCACATGATACTTACATATGGCTCTACAA
GTGTCATTTTTCTTCCATATTTGATATTATAAAAAATAAAATAAAAAACTAAGGAGATGATCCAGATAT
ATTGGAAAATGAAATGCAAAGGCTAAAAATAATTGAAATTAACATGAAATTAGTAAAAATTAC

SEQ.I.D. No. 16
Intron 2 antisense sequence (321 bp).

MMMVGCAAGCAATGCACCACAGTTAGTTTATATCAAAAAGAAGAAAGGTATTAACGGAGCTAAAAACTG
TTATATACCACATGAAAGAAGTTGATAATGTGAAAACACCATGCTCATAAAGATTGTAATTCAAATAAC
AAATGCCCACAGGAGTAAAGAGCTGTCTTTCCCAAGTTAAGGTATTATAAATTGGCGGAACGAAGTAAC
ACATGTTTGACATCTCCACACGGTGCACAGATCAAATATGCCATGAGCACCAGTCCAGAAGTTTTCCAA
CTATTTATATACTATCCATGCAACCATATAAATTATCAAACATAC

SEQ.I.D. No. 17
Intron 3 antisense sequence (504 bp).

CTGCAAAAAAAGAGAGCAGTTTACACAAGAAAAAACTGCTAAATCTCAACAAAAGTATCATGAATTTAA
TATTAAGGAAGCTATTTCGAACAGAAAGAGTAACTCATGATAATAGAAGGAAATTGTGAAGCAACAGAA
GGAAGACTTTCTTTATTTCTACAAAATTGCTTTAAGACTATATTTGATGCTTGTATAGTACATGTTGAA
TCCCCTCAGCTTCTTTATGTCTATACTTTTTTTATATTTTGAATCTCCTTAGTGAAAATCTTTGCTTTG
CCACTGACACTCCGGGGGTGTGTCACTTCTCCAAAAACCTTGTCTACTTTTTTGAAGACCCAATCAAAC
AGCTTTTTAAAAGATCAAAAAAATGGCCAGGTGCCACCTAAATGGAGCCACTACTTACTCCCCGGTATG
CAAAATTCTCTAGCAAAGTCAAAGTAGGTATAAACAATTCATCTTCCAAAATAAGGTCAAACTGCCTAA
AGCACAACTTTTGGCTGTTAC

SEQ.I.D. No. 18
Intron 4 antisense sequence (146 bp).

CTGCATTGTGGATGAGTTAATTAGAAGCATAACCTTAATAGCAATTAGAACATGTAAGAAAGCCAATGA
TGCTGCAACATCATGCTTTAATAGGAAAATCTGTTATGATGATGGAAACTACTATTTTGTAGTAGACGA
GGACCTAC

SEQ.I.D. No. 19
Intron 5 antisense sequence (218 bp).

CTGTTTAATTGCTGAAGTAGTAAGTTCTCAAGCACTTATAGAATTGACTCATTTTGTTAAGGGAAAGAG
TATGGGATCAAGTCCAAATTAGTAAAGACACAATTATTTTAACTTTTGCATTTCAAAATGTCTTACATA
ACAAGACTAGTAAGAACATGAATCGAAATGCCTGTGATGATGGTGTTCAAAATTCAGCTTCAAGGTATG
AATAACAAAAC

SEQ.I.D. No. 20
Intron 6 antisense sequence (198 bp).

CTGTATCCAGCAGACATAATAGGAGTGAACATAAAAATGTCACTGGATAAATAACTTATCATGATATTC
AGCGGCTACCAATATTCTGAAGGCCCATGGCGAAAATAAGTACTTTTATACTTTCAGGACGTATATATT
TGGATTCTATCTAACAATTGTTCTGAGAATTATTTAGTTGTAGAAATAAATTTAAAATAC

SEQ.I.D. No. 21
Intron 7 antisense sequence (208 bp).

CTGTGGTTAGAAGCTAAAAGTGAATAGATGAGAAAAATTACCTCCAAATAAGAGGGATATTGAAAAAGA
AACACAATGCATGAAAAGAATAAACAAATGATAAACGAGAAAATTGAATAATCCATCAGAACCCTGGTT
ACCTCACAAAGAGTGAGATTTTCCGTGGCTAACCTATATGAACCTTAAAATGCAATAGAAACAGACAAAC

SEQ.I.D. No. 22
Intron 8 antisense sequence (293 bp).

CTGTACAAGTTCATCAAACATTTCACAATTACTCCAAAACAGACACACTTGCAAACTCTATACAGTAAT
CTTCTATACTACAAAAAAGTAAACAATGTTTTTTTTAAGATGACATTTGTTCTCAGCAACATAATAGAA
ATCCCTAGACAATGGAAACATTCATCATGTTGTTTTCCTCTATGTTTCAACCCCTTTGATGTTCAACAG
TTCAGGTCATTTTGAGGAATGAATCTTGTTCAAGTAAGCCAAACTAATTGTAATTATCACAAAATATCT
AAAGATGTAAGACATAC

SEQ.I.D. No. 23
Intron 9 antisense sequence (376 bp).

CTGCATTTCATCATGAGGGGGAGGAAAGACGGAGAAATATAGATATCAGATTTAGACCATTTCAATTAG
TATCACTTCATTGTAAAGAAAAGGTAAGTATCCAACAAATATAGCAGGCTGTGGATTGGTAGCCTGAAA
CTATAGCTTCAAAGAATCAACTTAAGCTGCTCATCAAGGCCTTAGTGGTAGAAATGAGGCGGTAATAAG
TGTAAATGAATCTAATACTTGGATCTCGAAACAAAAATCAGAAATTCGGTTGGAAAATAAGTAGAACAA

GATGAAATGAGCTATCATCCCCAGAACCAAGTAGACTTCCAAGTAAGCAATCTAAAAATTACTAGATTA
TTTAACAAGCTGCGATTCAAAATACTTGAAC

SEQ.I.D. No. 24
Intron 10 antisense sequence (172 bp).

CTGCAAAGTGAAGTAACTAATCAGTACAGCTATTACCGAATTTGACCAGCTATTGGATTAAATAATATG
AAATCCATCATCAAGAAATGGAAGGTAAAAAGGTTTCTACTTGTCCTTGGATAGAATTAAAGCACTTCA
TAAACCCAACACTTTCAACTTTAGATGATTTTAC

SEQ.I.D. No. 25
Intron 11 antisense sequence (145 bp).

CTGTTTTCGTCATGCGAGGATCAGAAAAAAGAGTTAAATTAGACAATGTGAAAATGATTTGTTTCAGTT
ACTTCTCCATAAAACTTGTTCAGTACATTAAAAACAAGCAGAGCAATAATTTCATGGATAAGTAAAACA
TATATAC

SEQ.I.D. No. 26
Intron 12 antisense sequence (242 bp).

CTGTCATGAGAACAGATTGTATGTCAGCATGAAGACAAAGATCATCAATAAACAGTTTTCTCCTTTTTG
AATTAGCTAAACAACGCAGGGGGAGGGCAGGAGGCTCAAACACTTCCGAACTCAGACAGTCGGATATCT
TATACAACTAAAGATGGATGAGACAATTACAGTTCTTTTTGGTGAGAGAACTGTACCCTACATCTGTTA
TCTTATTATCAAAAGTTATTCAAGCAAATCCTTAC

SEQ.I.D. No. 27
Intron 13 antisense sequence (797 bp).

CTTCACAAACAAGGAGAAGAAGAAGCAAAAAGAAAGATGAATATAGTTAGCTTAGTTCAATATAAAAAA
TTTCTCTCCAAGCTATTTTTCTGCTAGCAAAATTCATTAGTTATTTAACTTTTCTATACATAAAGCTGC
ACAAAGAAATAGTAGTACATTTTTTTGACTTGCACAAAATAACTGTGTTGTCCATTTTCTGACATGTGT
TCATCTACATGCACTGTTTCAACAACAACAACTACTTCAGTCCCAAACAAGTTGGGTCGCTTTAGCTAC
ACATGTTGCTTTCACTTCTGTTACTTCTTTTTGGACTTTTTTTCTTGAGCCAAGGGTCTATTGAAAAAA
TCCTCTCTACCTCTGAGATAGGAGTAAGTTTTGCATACACTCTACCCTCCCCCTGAAACCACTTTGTGG
GACTACACGAGGTATGTTGTTGTTGATGTTAGCGCAGACACCAAAGGTGGACATTATATGACTATTCCT
AGCTTTACTTCAGGGCGGTTTTAAGTTCCCATCAACTTCATTTTTGATCATTTACCTAAGTTTATGCAG
GTGCAAGCTACATGCACTGGTTTAGGGAAAAAGAGGATAGAGAAGAATTTTTTTGGCATCCTTTTGTTT
TGTAACAGTAAGATGCCAAAAGTAGACCTTATTACGGCTATTCCTACCTTTCAAATTAGTAGTTCAGAG
GACTTAACTGGCGATTGTGGCGGTAATCAATAGTTAACTTCTATCGCATTCAAATAACTATGAACAAAA
CCACAATAAAAAGGGAGGTCACACGGCAAGAACTGTAC

SEQ.I.D. No. 28

Antisense DNA sequence of the SBE gene promoter region.

CGAATGGGTTTTGATAAAACTTTGAAATTAATTTCCATTGATTAAATTATGGTACTTTGC 60
TTCAGCTGCTGCCTTCTTGTATGTTCTGATTCTTGATTTCCTCATTTTAGTGGCTTTTTA 120
TAAAAAAACATTATGACCCTTTTGTTAGTCCTCCCCTTTCTGAATATTTCACTCAGACCC 180
CATTAGTTTCGAAATTGAAGTAAAACATATTTTTTTTAGTATTGTAGTTTTTTTATATTT 240
CTACTTACTTACTCGTTATACAATTTCTATTTAGGTAATCTAATCGACTTTTTGTATACA 300
TAATACATGTATTTTTGGTAAAGAGTTTTTTACTTTCTCCTAGTGGTAAGGCAGA TAG 360
TTAAGGATTTATTGACCTAATATGAACGCCAATAATTTTATATTTTGTATATACGTATAT 420
TTAAAAGTTTACTAGATATGTATAAATAAGATATTTAAAATTTAATTATAAATACAAATG 480
ATTATGGTAAAATTTTGACCTCCAAATTAAAATATTTAAAATCAAGATTTGTCACTACTT 540
ATATATATCTTGTTGTAAATCCCTTTTAATCAAGTTGTGAGTTTACAAATATTCGTTGGT 600
TAGGCTAAAAAAAAATAAGCTATAAAGATCAAGTATAAAATTATGCATTTTCTGCATTTAA 660
TTTGGAAAAATATGTTGGAGCAATCTAAAATTGTTTGTTGATTTATAAATAAGTCGTTTT 720
TTGTTTTTAATAATTGATAAACTATTTATTCTGCTTAAAGTTTTAGAATGTCAAAAAATA 780
ATTTATTTTAATGACCTTAAATGATTGAATAAGATGTAGACACACTCAATTACAAAGTT 840
CAATATTAATACACTTGTCTATTGGGTCATGGATTATATCATCTAATATAAATAACATGT 900
CAAATTAAAGCTTCTTATAAAGTTCATAGGAACTAAGATAAACTTTGTGAATGGCCAAGC 960
ATTTTTCAGAACATCATGGGTGGTATGACAATCAAATTGAACTTATGGGATGAAAAATGA 1020
ATATCATTCAACTAAGAGGGCACAACTTGACATGTTAGAAAGTAAAGCAAATTTAGTAGT 1080
GGGCCAAATAAAAGAAATTAATTTGTCAGTTTATTCTTAAACTTTACCTTCTTTGAACTT 1140
CCACGTTATCAAAGGTTCACGGTTCATATGAAGGCCATGTGTATCCTTTTTAATTTTGGT 1200
ATTCCGTGTTCAATATCGATTAATTTAAATTCGCATGACAAAATCCTATATTAAAGTATA 1260
AAGTATTTTCTAAAACAGACAAGTTCAATACTTTAATTTTACACTGAATGCATAAATTTA 1320
CACTATAATAATTCCAGTCGCAGTCTACATTACAATAATTAACAATTTTAGCATGAAATG 1380
AAAAACTTTAAATTATATGCCATCAAATCACTTAAAGTATACATTTTTTTAATAACTAGT 1440
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CLAIMS

1. A method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

2. A method according to claim 1 wherein starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

3. A method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

4. A method according to any one of claims 1 to 3 wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

5. A method according to any one of the preceding claims wherein the enzymatic activity is reduced or eliminated.

6. A method according to any one of the preceding claims wherein the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

7. A method according to any one of the preceding claims wherein the nucleotide sequence codes for all of at least one intron in a sense orientation.

8. A method according to any one of the preceding claims wherein the nucleotide sequence comprises the sequence shown as any one of SEQ.I.D. No. 1 to SEQ.I.D. No. 13 or a variant, derivative or homologue thereof, including combinations thereof.

9. A method according to any one of the preceding claims wherein the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

10. A sense sequence comprising the nucleotide sequence as defined in claim 8 or a variant, derivative or homologue thereof.

11. A promoter having a sequence shown as SEQ.I.D. No. 14, or a variant, derivative or homologue thereof.

12. A promoter according to claim 11 in combination with a gene of interest ("GOI").

13. A construct capable of comprising or expressing the invention according to any one of claims 10 to 12.

14. A vector comprising or expressing the invention according to any one of claims 10 to 13.

15. A combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

16. A cell, tissue or organ comprising or expressing the invention according to any one of claims 10 to 15.

17. A transgenic starch producing organism comprising or expressing the invention according to any one of claims 10 to 16.

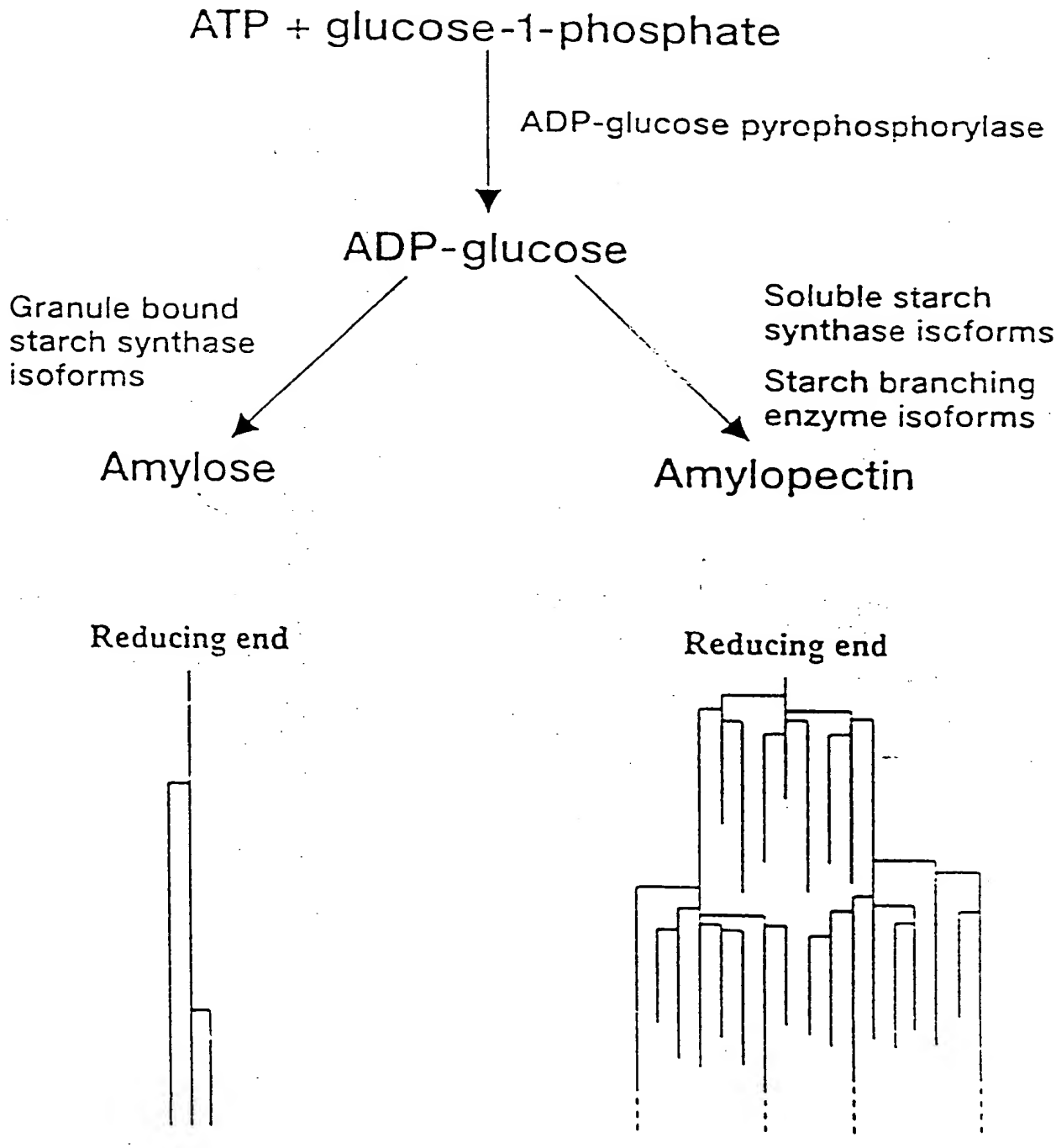
18. A transgenic starch producing organism according to claim 17 wherein the organism is a plant.

19. A starch obtained from the invention according to any one of the preceding claims.

20. pBEA11 (NCIMB 40754).

21. An intron nucleotide sequence that is obtainable from λ-SBE 3.2 (NCIMB 40751) or λ-SBE 3.4 (NCIMB 40752) or a variant, derivative or homologue thereof.

22. A method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence; wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.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ADP-glucose pyrophosphorylase
ADP-glucose
Granule bound starch synthase isoforms
Soluble starch synthase isoforms
Starch branching enzyme isoforms
Amylose
Amylopectin
Reducing end
Reducing end

Fig 1

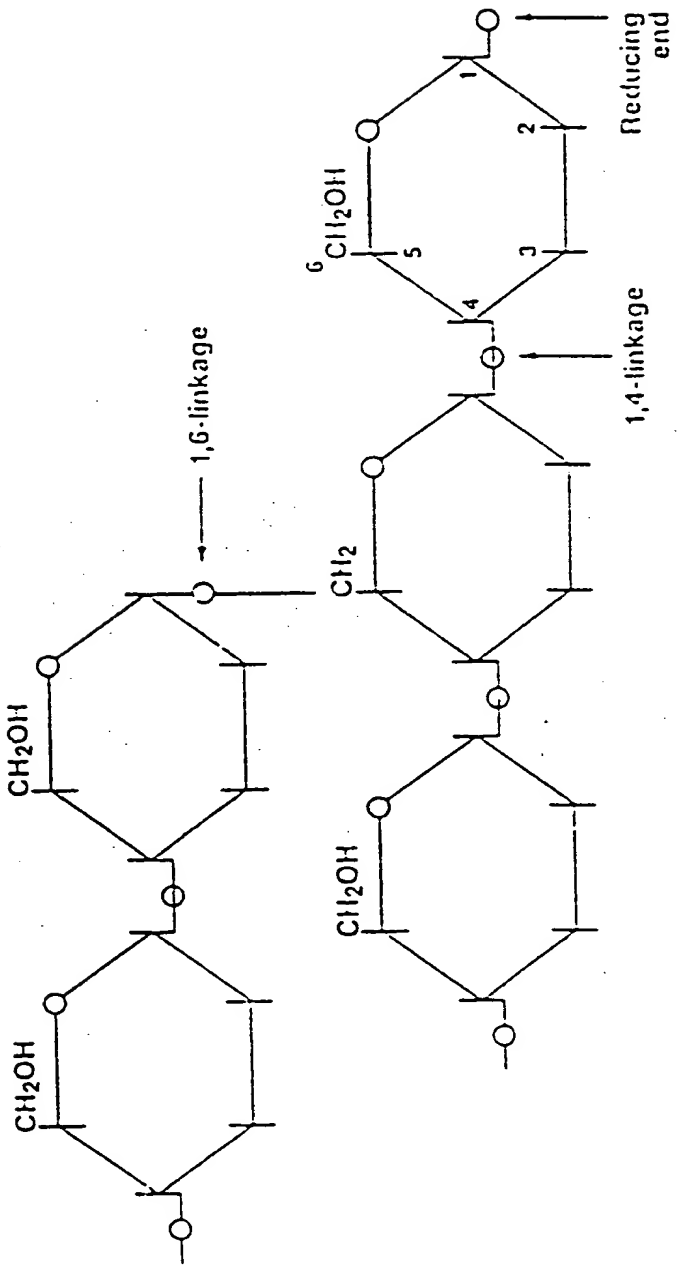

Reducing end
1,4-linkage
1,6-linkage
CH_2OH
CH_2OH
CH_2OH
CH_2
CH_2OH
1
2
3
4
5
6

Fig 2

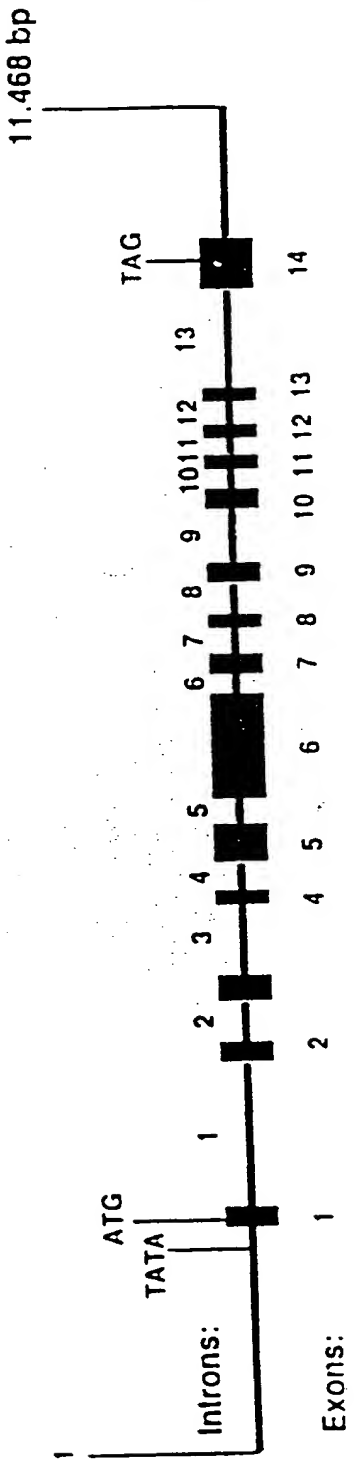
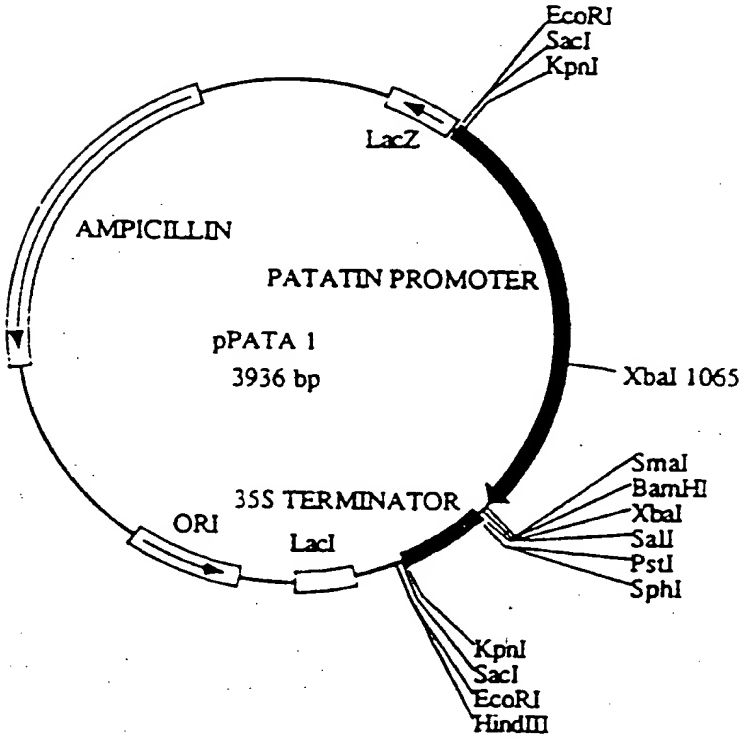


Fig 3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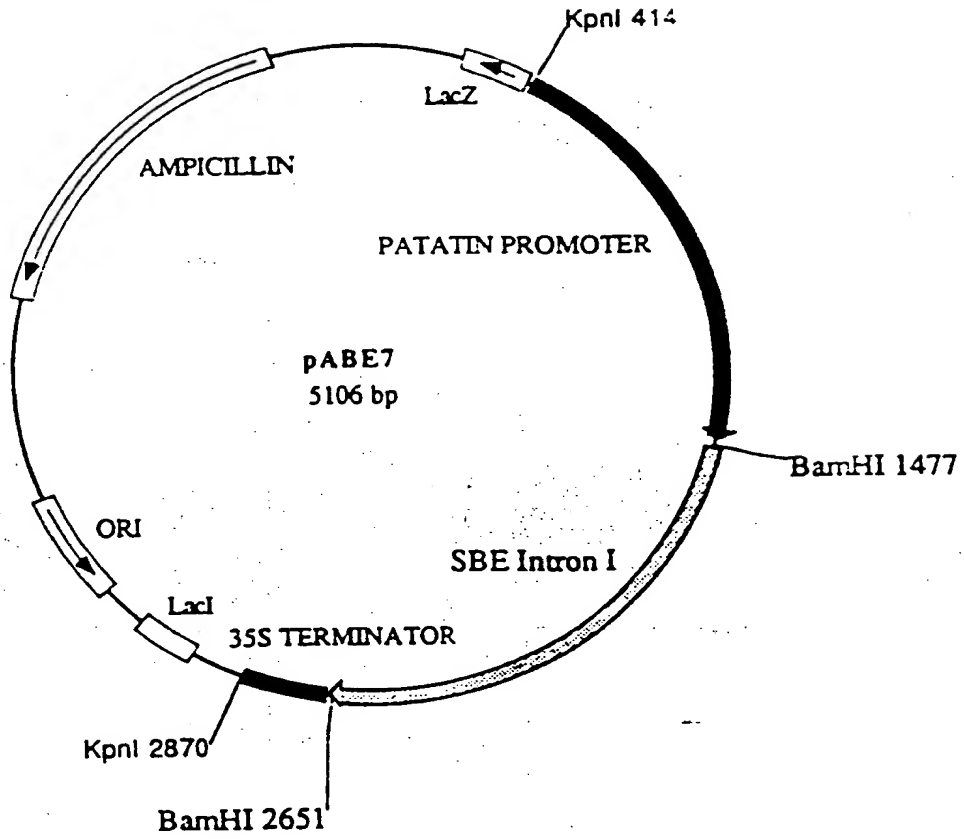
SacI
KpnI
LacZ
AMPICILLIN
PATATIN PROMOTER
pPATA 1
3936 bp
XbaI 1065
SmaI
BamHI
XbaI
SalI
PstI
SphI
35S TERMINATOR
ORI
LacI
KpnI
SacI
EcoRI
HindIII

Fig 4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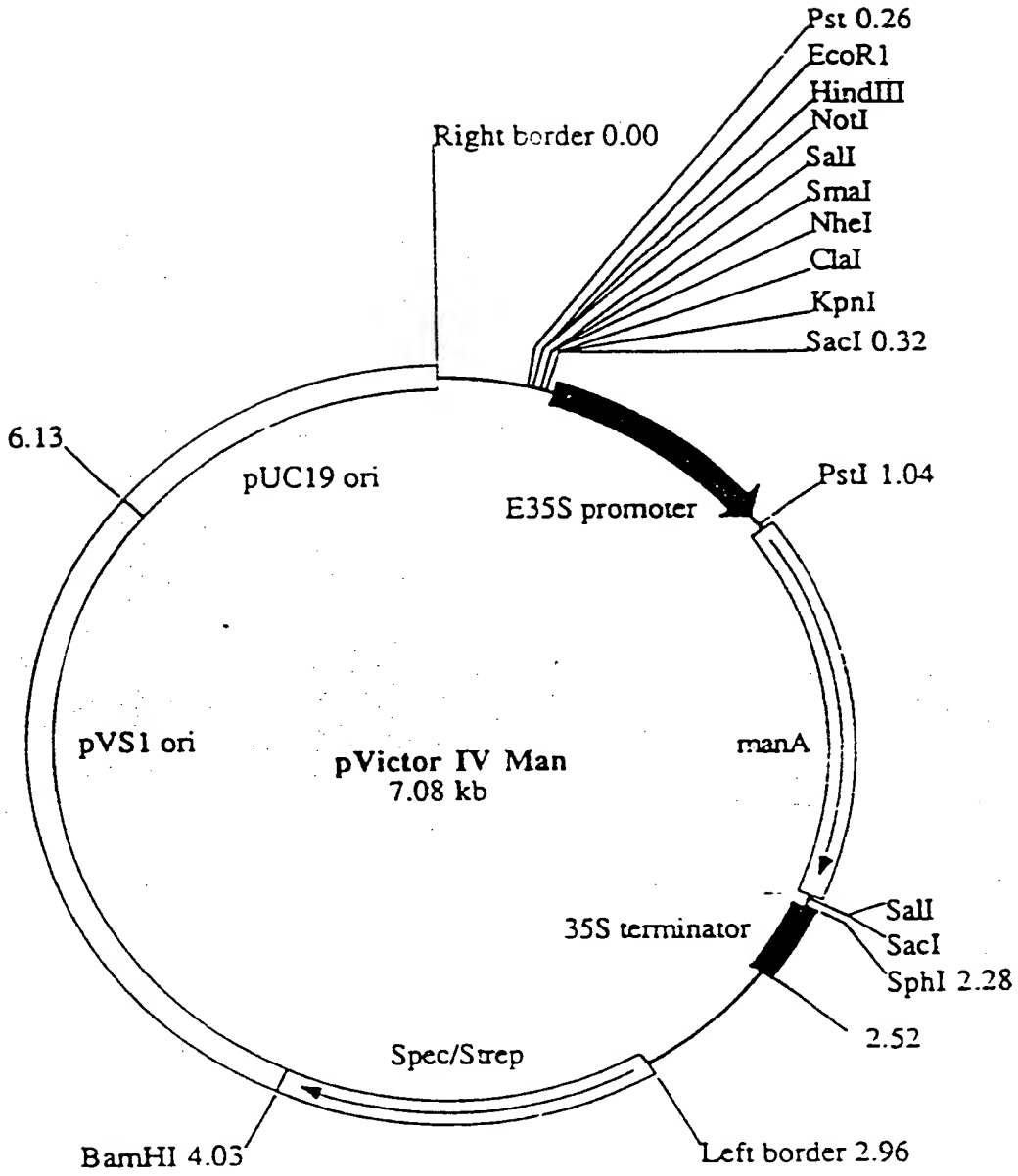
LacZ
AMPICILLIN
PATATIN PROMOTER
pABE7
5106 bp
BamHI 1477
ORI
SBE Intron I
LacI
35S TERMINATOR
KpnI 2870
BamHI 2651

Fig 5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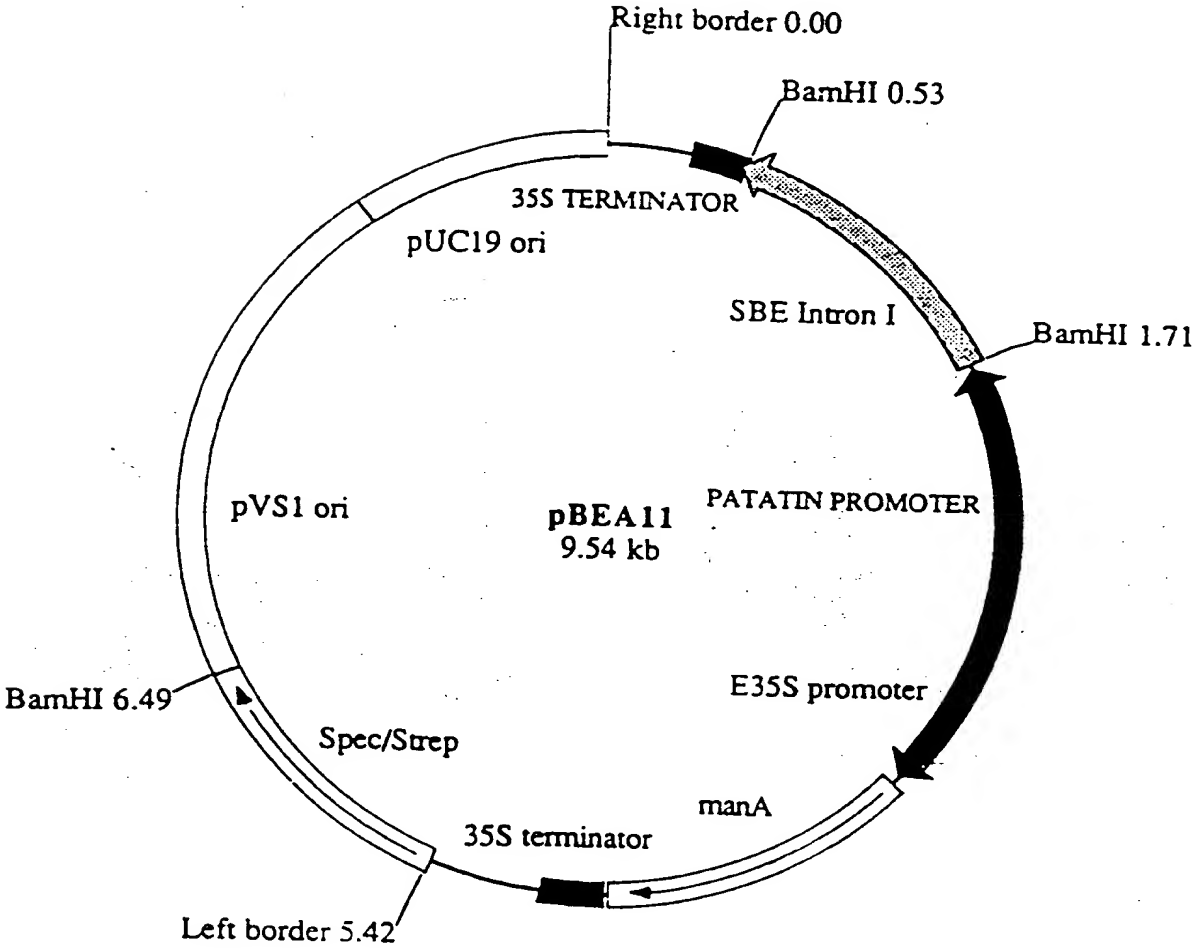
EcoR1
HindIII
NotI
SalI
SmaI
NheI
ClaI
KpnI
SacI 0.32
Right border 0.00
6.13
pUC19 ori
E35S promoter
PstI 1.04
pVS1 ori
pVictor IV Man
7.08 kb
manA
SalI
SacI
SphI 2.28
35S terminator
2.52
Spec/Strep
BamHI 4.03
Left border 2.96

Fig 6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BamHI 0.53
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pUC19 ori
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pVS1 ori
pBEA11
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PATATIN PROMOTER
BamHI 6.49
E35S promoter
Spec/Strep
manA
35S terminator
Left border 5.42

Fig 7

```
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CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTTTCAAACTCTT     180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTTTCCTCAATGATGTTTACAGTGTTGTG     240
TTGTCATCTGTACTTTTGCCTATTACTTGTTTTGAGTTACATGTTAAAAAAGTGTTTATT     300
TTGCCATATTTTGTTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA     360
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AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG     480
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AACGTTAATTTAGTAATTTTTGTTACGTACGTATATGAAATATTGAATTAATCACATGAA     600
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AAAATACTTTATACTTTAATATAGGATTTTGTCATGCGAATTTAAATTAATCGATATTGA     960
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GAATGATATTCATTTTTCATCCCATAAGTTCAATTTGATTGTCATACCACCCATGATGTT    1200
CTGAAAAATGCTTGGCCATTCACAAAGTTTATCTTAGTTCCTATGAACTTTATAAGAAGC    1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA    1320
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AAAATAAATTATTTTTTGACATTCTAAAACTTTAAGCAGAATAAATAGTTTATCAATTAT    1440
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Fig 8

```
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TACCATAATCATTTGTATTTATAATTAAATTTTAAATATCTTATTTATACATATCTAGTA  1740

AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTCATATTAGGTCAATA  1800

AATCCTTAACTATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA  1860

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AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAAATATGTTTTACTTCAATTTCG  1980

AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT  2040

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                                    M  E  I  N  F  K  V  L  S  K
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 P  I  R  G  S  F  P  S  F  S  P  K  V  S  S  G
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Fig 8 continued.

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                    A  S  R  N  K  I  C  F  P  S  Q  H  S  T  G
ACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGAGT                    3480
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 H  R  M  K  R  Y  V  D  Q  K  M  L  I  E  K  Y  E  G  P  L
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 E  E  F  A  Q  G
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Fig 8 continued

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 N  R  E  D  G  C  I  V  Y  R  E  W  A  P  A  A  Q
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ttgcagcatcattggctttcttacatgttctaattgctattaaggttatgcttctaatta          4740

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V  W  V  D  R  I  P  A  W  I  K  Y  A  T  A  D  A  T  K  F
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GAGCCCCACGAATCTATGAAGCACATGTCGGCATGAGCAGCTCTGAGCCACGTGTAAATT          5340
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  Q  L  M  A  I  M  E  H  S  Y  Y  G  S  F  G  Y  H  V  T  N
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  F  F  A  V  S  S  R  Y  G  N  P  E  D  L  K  Y  L  I  D  K
AAGCACATAGCTTGGGTTTACAGGTTCTGGTGGATGTAGTTCACAGTCATGCAAGCAATA          5580
  A  H  S  L  G  L  Q  V  L  V  D  V  V  H  S  H  A  S  N  N
ATGTCACTGATGGCCTCAATGGCTTTGATATTGGCCAAGGTTCTCAAGAATCCTACTTTC          5640
  V  T  D  G  L  N  G  F  D  I  G  Q  G  S  Q  E  S  Y  F  H
ATGCTGGAGAGCGAGGGTACCATAAGTTGTGGGATAGCAGGCTGTTCAACTATGCCAATT          5700
  A  G  E  R  G  Y  H  K  L  W  D  S  R  L  F  N  Y  A  N  W
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  E  V  L  R  F  L  L  S  N  L  R  W  W  L  E  E  Y  N  F  D
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  L  M  L  A  N  N  L  I  H  K  I  F  P  D  A  T  V  I  A  E
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Fig 8 continued

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                                                  S  I  V  G
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Fig 8 continued

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                                                   V  V  V  F
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  A  L  D  S  D  A  W  E  F  G  G  H  G  R
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                                                   T  G  H  D  V
TGACCATTTCACATCACCAGAAGGAATACCTGGAGTTCCAGAAACAAATTTCAATGGTCG                8400
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Fig 8 continued

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ttattttgtgcaagtcaaaaaaatgtactactatttctttgtgcagctttatgtatagaa                  9120

aagttaaataactaatgaattttgctagcagaaaaatagcttggagagaaatttttttata                 9180

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GCTTATTACAGAGTTGATGAACGCATGTCAGAAACTGAAGATTACCAGACAGACATTTGT                  9300
A  Y  Y  R  V  D  E  R  M  S  E  T  E  D  Y  Q  T  D  I  C
AGTGAGCTACTACCAACAGCCAATATCGAGGAGAGTGACGAGAAACTTAAAGATTCGTTA                  9360
S  E  L  L  P  T  A  N  I  E  E  S  D  E  K  L  K  D  S  L
TCTACAAATATCAGTAACATTGACGAACGCATGTCAGAAACTGAAGTTTACCAGACAGAC                  9420
S  T  N  I  S  N  I  D  E  R  M  S  E  T  E  V  Y  Q  T  D
ATTTCTAGTGAGCTACTACCAACAGCCAATATTGAGGAGAGTGACGAGAAACTTAAAGAT                  9480
I  S  S  E  L  L  P  T  A  N  I  E  E  S  D  E  K  L  K  D
TCGTTATCTACAAATATCAGTAACATTGATCAGACTGTTGTAGTTTCTGTTGAGGAGAGA                  9540
S  L  S  T  N  I  S  N  I  D  Q  T  V  V  V  S  V  E  E  R
GACAAGGAACTTAAAGATTCACCGTCTGTAAGCATCATTAGTGATGTTGTTCCAGCTGAA                  9600
D  K  E  L  K  D  S  P  S  V  S  I  I  S  D  V  V  P  A  E
TGGGATGATTCAGATGCAAACGTCTGGGGTGAGGACTAGTCAGATGATTGATCGACCCTT                  9660
W  D  D  S  D  A  N  V  W  G  E  D
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AATTTGCATGATAAAAAGTCTGATTTTATGATCGCTATCCTCGCTCTCTGAGAAAGAAGC                  9780

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CCAGTGTCAATACACAACCTAGGCCAACATCGAAAGCATAACTGTAAACTCATGCATGAA                 10260

GAAATCAGTCGTAAAAATGAATAAATGCGACATAAAAACAAATTGCATGTATCATTAATG                 10320

TGACTTAACTACAAGTAAAAATAAATTTAACAAATGTAACTTAACTACAAGTAAAAATAA                 10380

ATTGCTTCTATCATTAACAAACAAACAGAATTAAAAAGAAAAAAACATACTAAATCTTAC                 10440

CGTCATTCGATAAAAAAAAATACCAAATTCATAATGCAAGGAAAACGAAACGCGTCCTGA                 10500
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Fig 8 continued

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         10        20        30        40        50        60
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CAAAAAAAAGAACACGATCCTTTGCACCCGTTCGATGATTATCAGTATGTTCACAAAAAA  10620
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CCAATTTATCTTATTCTTATCTGCCACAAAATAATCGGTTTCACACTATTCTCTTGTTAT  10740
ACAAAATTGACAAGTAGGAAGGAGAGGAGTCATCCAAATAAACGGTGCACGTTCTTTGAG  10800
AAAAGTCTTATTTTTCGTAAGATCCAATTTCAACAAACTTTTCTTCAAGTCAAAATTCCT  10860
GATAGTGTATCTCCTCTCGACGACCTCTTGCATTGAACGATCTCCGCTTATCATGAAAAG  10920
TTGCTTGGATAACAAGTATTGCAAGGGGGGGACAGTAGCTATTAAGTTAGTCGGCCCAAG  10980
GAAATGGAGGAGTGATAGTCTCGAATATTATTCACCTCTTTAGCATTACCCGGTCTGGCT  11040
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TCGCGAGTTGTGGAAGGTTCAAGTTACTCGATTCGTGATTTTCAAGTATGAGTGGTGAGA  11160
GAGATTCGATATTTTCACGAGGTGTATTCGAGGTCTAGTAGAACGAAGGGTGTCACTAAT  11220
GAAAGTTTCAAGAGTTCATCATCATCTTCTTCTAGTAGATTTTCGCTTTCAAATGAGTAT  11280
GAAAATTCTTCCTCTTTTCTATTGATTTTCTTCATTGTTTTCTTCATTGTTGTGGTTGTT  11340
ATTGAAAAGAAAGAAAATTTATAACAGAAAAAGATGTCAAAAAAAAGGTAAAATGAAAGA  11400
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TCAGCTAAGTTAGAATTC                                             11478
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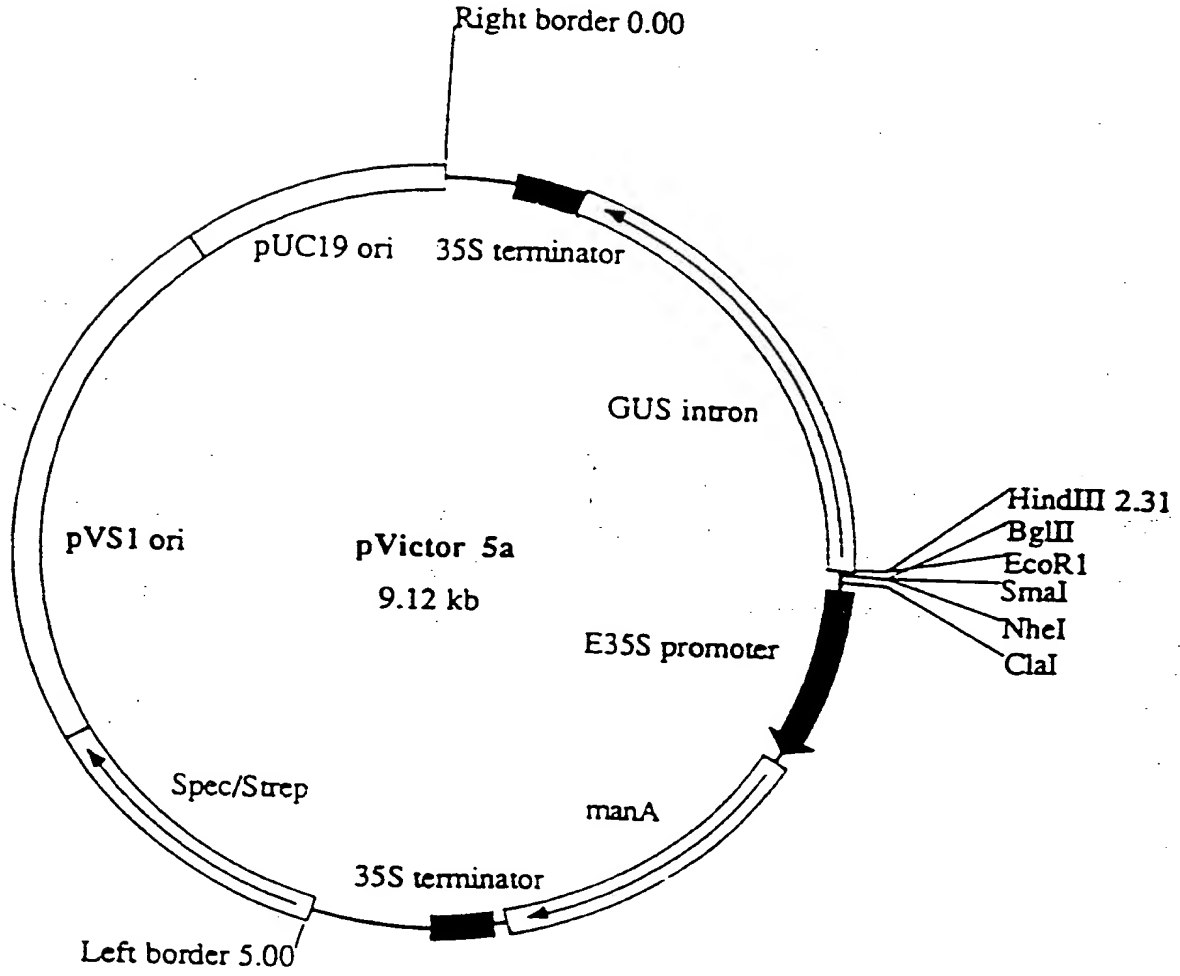
Fig 8 continued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35S terminator
GUS intron
HindIII 2.31
BglII
EcoR1
SmaI
NheI
ClaI
pVS1 ori
pVictor 5a
9.12 kb
E35S promoter
Spec/Strep
manA
35S terminator
Left border 5.00

Fig 9

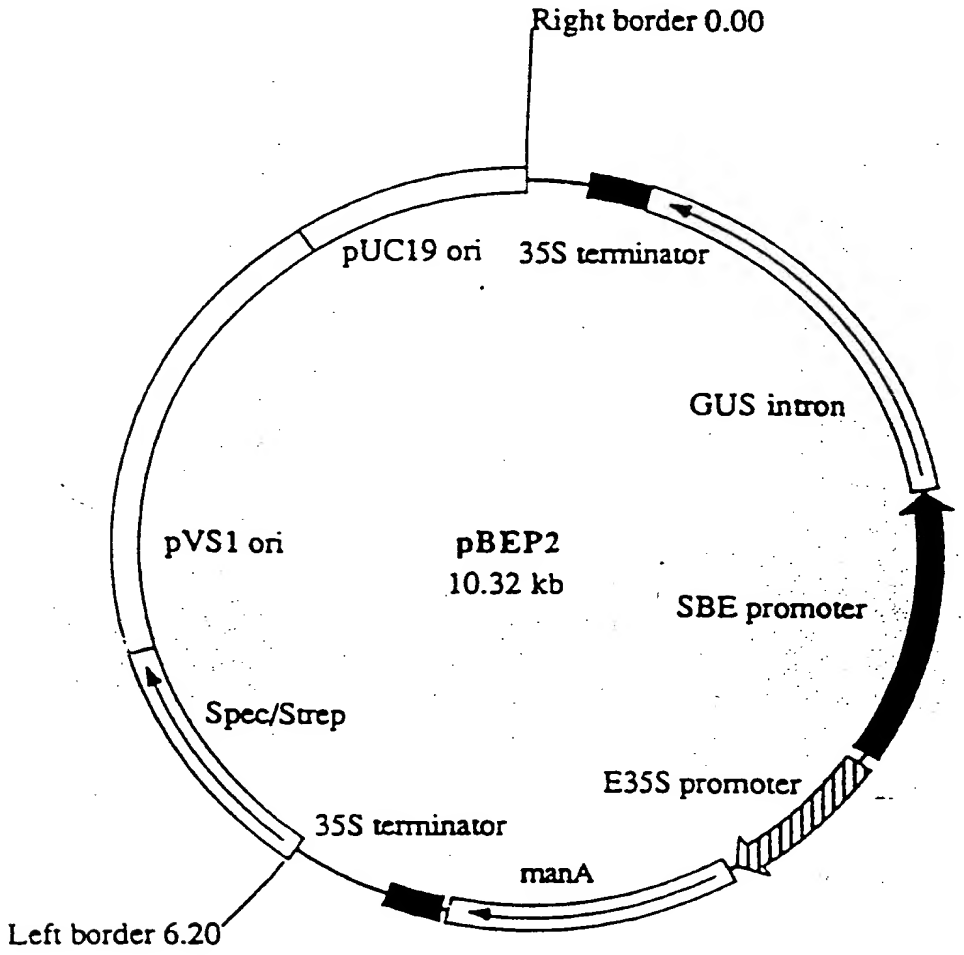
Right border 0.00
pUC19 ori
35S terminator
GUS intron
pVS1 ori
pBEP2
10.32 kb
SBE promoter
Spec/Strep
E35S promoter
35S terminator
manA
Left border 6.20

Fig 10

Applicant's or agent's file reference number	International application No.
	PCT/EP 96 / 03053

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 19, lines 18 to 27

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depositary institution

The National Collections of Industrial and Marine Bacteria Limited (NCIMB)

Address of depositary institution *(including postal code and country)*

23 St. Machar Drive
Aberdeen
Scotland
AB2 1RY
United Kingdom

Date of deposit	Accession Number
13 July 1995	NCIMB 40754, NCIMB 40751, NCIMB 40752

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

In respect of those designations in which a European patent is sought, and any other designated state having equivalent legislation, a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer: L.R. Pether	Authorized officer

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

PCT/EP 96 / 03053

Danisco Biotechnology
gebrogade 1
P O Box 17
DK-1001 Copenhagen K
Denmark

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Escherichia coli DH5α-pBEA11	NCIMB 40754

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 21 July 1995 Terence Dando

[1] Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

PCT/EP96/03053

Danisco Biotechnology
Langebrogade 1
P O Box 17
DK-1001 Copenhagen K
Denmark

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40754 Date of the deposit or of the transfer: 13 July 1995

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 13 July 1995 [2]. On that date, the said microorganism was

[X] [3] viable

[ ] [3] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: NCIMB Ltd Address: 23 St Machar Drive Aberdeen Scotland UK AB2 1RY	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Terence Dando Date: 21 July 1995

[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DR A Buchter-Larsen
Danisco Biotechnology
Langebrogade 1
P O Box 17
DK-1001 Copenhagen
Denmark

INTERNATIONAL FORM **PCT/EP 96 / 03053**

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this pag

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Bacteriophage λEMBL3 SP6/T7 λSBB3.2	NCIMB 40751

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on . (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 20 July 1995

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Dr A Buchter-Larsen
Danisco Biotechnology
Langebrogade 1
P O Box 17
DK-1001 Copenhagen K
Denmark

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40751 Date of the deposit or of the transfer: 19 July 1995

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 19 July 1995 [2]. On that date, the said microorganism was

[3] [X] viable

[3] [ ] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

Address: NCIMB Ltd
23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 20 July 1995

[4] Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDUR

Dr A Buchter-Larsen
Danisco Biotechnology
Langebrogade 1
Box 17
DK-1001 Copenhagen
Denmark

INTERNATIONAL FORM **PCT/EP 96 / 03053**

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
Issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

NAME AND ADDRESS
OF DEPOSITOR

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Bacteriophage λEMBL3 SP6/T7 λSBE3.4	NCIMB 40752

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

[ ] a scientific description

[X] a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 13 July 1995 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on . (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: **NCIMB Ltd**

Address: 23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 20 July 1995

1 Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.

Form BP/4 (sole page)

PCT/EP96/03053

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

Dr A Buchter-Larsen
Danisco Biotechnology
Langebrogade 1
P O Box 17
DK-1001 Copennagen K
Denmark

INTERNATIONAL FORM

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: As above Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40752 Date of the deposit or of the transfer: 13 July 1995

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 19 July 1995 [2]. On that date, the said microorganism was

[3] [X] viable

[3] [ ] no longer viable

[1] Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

[2] In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

[3] Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:

Address: NCIMB Ltd
23 St Machar Drive
Aberdeen Scotland
UK AB2 1RY

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

Date: 20 July 1995

[4] Fill in if the information has been requested and if the results of the test were negative.